

**AUP Section:** Animal Disposition

**Species:** Xenopus spp.

**Methods of euthanasia:** (select all that apply)

**NOTE:** Chemical methods of euthanasia alone may require up to 3-5 hours before death results. A physical means of ensuring euthanasia, noted below under secondary methods, must be applied once unconsciousness is achieved.

**Primary methods:**

Agent/Method Name	Dosage (in mg/kg if possible)	Route
Buffered Tricaine methanesulfonate (MS-222)	2-5 g/L + sodium bicarbonate	Immersion
Benzocaine hydrochloride	≥250mg/L	Immersion
Benzocaine hydrochloride	2 cm × 1 mm strip (wet weight, 100 mg) of 20% benzocaine gel	Topical
Pentobarbital sodium + Phenytoin (pentobarbital-based euthanasia solution)	≥1100 mg/kg sodium pentobarbital with sodium phenytoin (≥0.3 mL solution per frog)	IV or Intracoel mic injection

**Secondary methods:**

Agent/Method Name	Dosage (in mg/kg if possible)	Route
Maceration (for non-transgenic animals; indicate instrument utilized)	Not applicable	Not applicable
Exsanguination or vital tissue harvest (inclusive of heart and/or lungs and/or brain)	Not applicable	Not applicable
Decapitation and double pithing (indicate instrument utilized)	Not applicable	Not applicable

**Description of Euthanasia Procedure:** (select all that apply):

Procedure	Description
<u>Tricaine or benzocaine immersion (all ages)</u>	<p><u>Euthanasia will be carried out as described in the most current version of the AVMA Guidelines for the Euthanasia of Animals.</u></p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> <li>1. Immerse frogs in a solution of tricaine methanesulfonate benzocaine hydrochloride.</li> <li>2. The solution should be buffered with sodium bicarbonate to a pH of 7.0-7.5.</li> <li>3. Frogs should remain in the solution for at least 10 minutes following loss of righting reflex and withdrawal response (adults) or following opercular movement (embryos/larvae)</li> <li>4. Perform a secondary method of euthanasia <ol style="list-style-type: none"> <li>a. Adults: double pith or exsanguination</li> <li>b. Embryos/larvae: bleach (1 part sodium hypochlorite 6.15% to 5 parts water) or rapid freeze</li> </ol> </li> </ol>
<u>Benzocaine topical gel (adults)</u>	<p>Euthanasia will be carried out as described in the most current version of the <a href="#">AVMA Guidelines for the Euthanasia of Animals</a>.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> <li>1. Gently manually restrain frog. Personnel should wear wet gloves.</li> <li>2. Apply benzocaine gel directly to the ventral abdomen of the frog.</li> <li>3. Return frog to a wet bucket/tank without water until deep anesthesia is confirmed via loss of righting reflex and withdrawal response</li> <li>4. Frogs should remain undisturbed in the bucket or tank for at least 10 minutes following loss of righting reflex and withdrawal response</li> <li>5. Perform secondary method of euthanasia: double pith or exsanguination</li> </ol>
<u>Injection of Pentobarbital Euthanasia Solution (adults)</u>	<p>Euthanasia will be carried out as described in the most current version of the <a href="#">AVMA Guidelines for the Euthanasia of Animals</a>.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> <li>1. Gently manually restrain frog. Personnel should wear wet gloves.</li> <li>2. Inject <math>\geq 0.3</math> mL pentobarbital solution per frog intracoelomically or via the dorsal lymph sac.</li> <li>3. Return frog to a wet bucket/tank without water until deep anesthesia is confirmed via loss of righting reflex and withdrawal response.</li> </ol>

	<ol style="list-style-type: none"><li>4. Frogs should remain undisturbed in the bucket or tank for at least 10 minutes following loss of righting reflex and withdrawal response.</li><li>5. Perform a secondary method of euthanasia: double pith or exsanguination</li></ol>
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**Details for Carcass Disposal:**

Ova and embryos will be collected in a sieve and prevented from going down the drain. All carcasses and reproductive material will be frozen for incineration or another approved disposal method by OLAC.

**References:**

American Veterinary Medical Association. 2020. AVMA Guidelines on Euthanasia, 2020 update.

[Journal of the American Association for Laboratory Animal Science](#) 2009 Vol. 48 No. 5 pp. 512-516

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